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Sperm Morphology Assessment in the Era of Intracytoplasmic Sperm Injection: Reliable Results Require Focus on Standardization, Quality Control, and Training

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Semen analysis is the first, and frequently, the only step in the evaluation of male fertility. Although the laboratory procedures are conducted according to the World Health Organization (WHO) guidelines, semen analysis and especially sperm morphology assessment is very difficult to standardize and obtain reproducible results. This is mainly due to the highly subjective nature of their evaluation. ICSI is the choice of treatment when sperm morphology is severely abnormal (teratozoospermic). Hence, the standardization of laboratory protocols for sperm morphology evaluation represents a fundamental step to ensure reliable, accurate and consistent laboratory results that avoid misdiagnoses and inadequate treatment of the infertile patient. This article aims to promote standardized laboratory procedures for an accurate evaluation of sperm morphology, including the establishment of quality control and quality assurance policies. Additionally, the clinical importance of sperm morphology results in assisted reproductive outcomes is discussed, along with the clinical management of teratozoospermic patients.

Keywords: Abnormality, teratozoospermia; Morphology, stain; Sperm

INTRODUCTION

Infertility is defined as the inability to achieve a successful pregnancy after 12 months or more of regular, unprotected sexual intercourse [1]. The World Health Organization (WHO) demographics estimate that about 200 million people are affected by infertility globally [2,3], with male factor contributing to 50% of infertility cases [4]. A meta-analysis published in 1992 reported a decline in sperm quality over a period of 50 years (1938 to 1991) [5] while, more recently, Levine et al [6] reported a 50% to 60% decline in sperm count from 1973 to 2011 across North America, Europe, New Zealand, and Australia. Furthermore, other researchers have reported a significant decline in sperm concentration, total count, morphology, and motility between 2000 to 2017 [7]. A negative association is also reported between poor morphology and gonadotropins [8]. As the analysis of sperm parameters is the primary approach for identifying and diagnosing male infertility, semen analysis is of particular importance since it defines fertility status and potential, as well as the course of natural or assisted reproduction [9]. In this framework, sperm morphology is established as the most prominent component, as this parameter cannot be surpassed even by the most invasive interventions such as intracytoplasmic sperm injection (ICSI).

Interestingly, there have been significant changes in the classification of sperm morphology over the years. Normal sperm morphology reference values have been revised dramatically from ≥80.5% reported in the 1st edition of the WHO manual [10,11] to ≥14% in the 4th edition [12], and even lower to ≥4% in the 5th and most recent edition [13]. The 'strict criteria' for assessing sperm morphology classify semen as normal if the percentage of normal sperm is ≥14% [14,15]. Based on this classification, abnormal sperm morphology has been associated with poor fertilization and clinical outcomes after assisted reproductive technology (ART), thereby establishing sperm morphology as a predictor of ART outcomes [16-18]. The cut-off for sperm morphology based on strict criteria was further revised in the 5th edition of the WHO manual [13] which uses the 5th centiles (and their 95% confidence intervals [CIs]) as the lowest reference limit (≥4% cutoff for normal sperm morphology). The lowest limit for morphologically normal forms of spermatozoa is 4% (95% CI, 3%–4%) and all borderline forms are considered abnormal. This classification serves as a surrogate tool to choose the most appropriate type of ART procedure for infertile couples [19]; if the percentage of normal forms is ≥4%, the probability of fertilization with ART is high and procedures such as intrauterine insemination (IUI) or *in vitro* fertilization (IVF) may be selected. In specimens with otherwise normal count and motility, poor morphology may itself be a determinant in deciding whether or not to proceed with ICSI. When the percentage of sperm with normal morphology is <4%, fertilization with IUI and IVF is poor, and ICSI should be preferred [20].

The predictive value of sperm morphology in ART continues to be a matter of debate. Recent studies sug-

gest that morphology may not be a good predictor of fertilization or pregnancy outcome in ICSI [21-25]. Abnormal fertilization following ICSI has been associated with abnormal parameters in semen analysis. Oligoasthenozoospermia and oligoasthenoteratozoospermia have been suggested to be further associated with lower cleavage and blastocyst formation rates [26]. A careful assessment is critical to identify the underlying cause of infertility, as this can serve as a tool to choose the most appropriate type of ART method [27]. However, heterogeneity of study groups, differences in staining methods, intra- and inter-laboratory variations, differences in the scoring classifications, and manual *versus* computer assisted semen analyzer (CASA) scoring may be among the many contributory factors for the lack of robust predictive power [19,28,29].

Diagnostic and predictive value of sperm morphology can be improved by uniform and consistent application of the defined strict criteria for scoring sperm morphology, standardization of the staining methods, internal and external quality controls (QCs), and training of the laboratory personnel [11,19,30].

In this review, we aim to: a) summarize standardized laboratory procedures for a proper evaluation of sperm morphology; b) highlight the importance of QC and quality assurance (QA) in laboratory assessment of sperm morphology; c) discuss the association between abnormal sperm morphology (teratozoospermia) and ART outcomes; and d) review the clinical management of men with abnormal sperm morphology.

MORPHOLOGICAL ASSESSMENT OF SPERM BY IMPLEMENTATION OF STANDARDIZATION AND QUALITY CONTROL

1. Preparation of semen sample

Semen sample is collected in a sterile collection container according to the recommendations provided by the WHO (2010) and incubated at 37°C for 30 minutes to allow liquefaction [13]. If the ejaculate is viscous, proteolytic enzymes such as α-chymotrypsin or bromelain can be added to the sample and incubated at 37°C for an additional 10 minutes [13,31]. The liquefied sample is vortexed for 10 seconds, and a 10 μL aliquot is quickly removed. If the sperm concentration is $<2\times10^6$/mL, the sample is centrifuged at 600 *g* for 10 minutes to remove most of the supernatant, leaving about 100 μL of seminal plasma in the underlying concentrate. The pellet is resuspended by gentle pipetting to redilute the sample, not exceeding 50×10^6/mL. Centrifugation may affect sperm morphology and should be recorded in the patient's worksheet [32].

2. Preparing a smear for sperm morphology

On a clean frosted slide with the patient identifiers, 10 μL of well-mixed semen aliquot is added on one end of the slide. A second slide is used with an angle of 45° to quickly spread the drop of semen along the frosted slide, forming a smooth and even smear. The slides are prepared in duplicate and then air-dried before staining.

The smear is stained using a rapid stain such as Diff-Quik, which consists of a fixative (triarylmethane dye, methanol), solution I (xanthene dye, sodium azide, pH buffer), and solution II (thiazine dye, pH buffer) (Fig. 1). According to the Diff-Quik staining protocol, once the smear slide is dry, it is immersed in the fixative five times and allowed to dry completely for 15 minutes. Once dried, the slide is immersed three times in solution I for 10 seconds, following which the excess stain is drained, and the slide is immersed five times in solution II for 10 seconds. The excess stain is quickly rinsed in sterile water, and the slide is placed vertically on absorbent paper to dry. Finally, a few drops of the mounting medium Cytoseal are placed on the slide, and the slide is covered with a coverslip. The slide is allowed to dry completely before it is examined under a bright field microscope [33]. The stained smear is exam-

Fig. 1. Main components of the Diff-Quik staining method: fixative, Solution I, Solution II, and water to rinse excess stain.

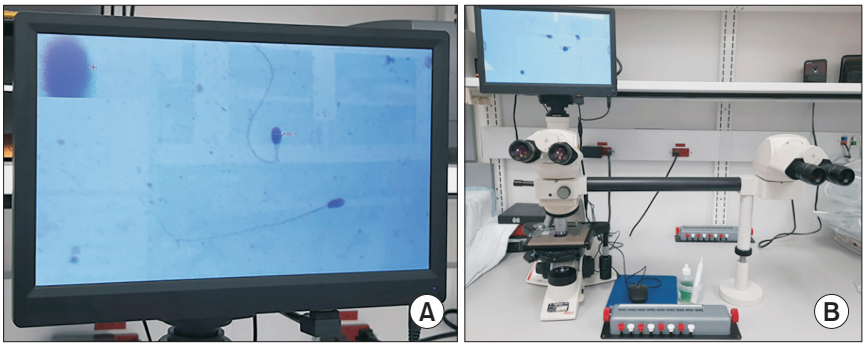
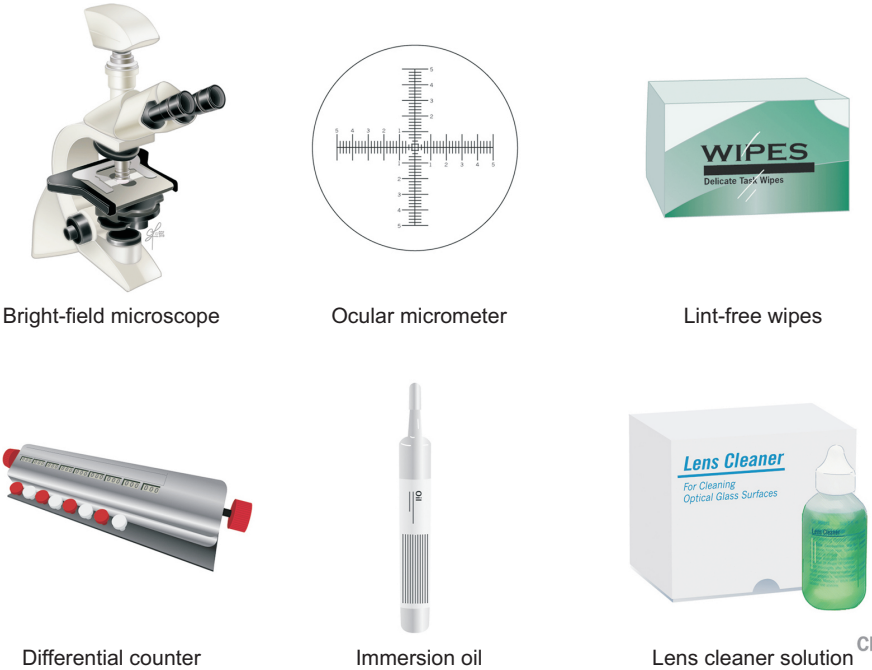


Fig. 2. (A) A magnified image showing sperm morphology staining as seen on an external monitor. (B) Microscope equipped with 100× oil-immersion brightfield objective used for the analysis of sperm morphology.



Bright-field microscope | Ocular micrometer | Lint-free wipes

Differential counter | Immersion oil | Lens cleaner solution

Fig. 3. Supplies and instruments used for the evaluation of sperm morphology.

ined using a bright field microscope with 100× objective and 10× eyepiece (Fig. 2). For optimum sharpness, the immersion oil used at 100× magnification should have a refractive index (RI) of 1.52, similar to that of glass and the cells being examined. An ocular micrometer is placed in one of the microscope's eyepieces to accurately measure the sperm dimensions (Fig. 3) [13]. Based on the strict criteria, a spermatozoon must conform to all normal morphological criteria as outlined above. Without the aid of an ocular micrometer, a precise evaluation of morphology cannot be performed [13].

3. Evaluation of sperm morphology

Evaluation of sperm morphology is highly subjective and depends largely on the perception of the observer scoring the slide. To obtain reliable and reproducible results, the andrology laboratory must develop a detailed step-by-step protocol. In addition, the use of an ocular micrometer is essential to measure the sperm dimensions. As reported in the WHO guidelines, the sperm head should be oval in shape, smooth, and regularly contoured, 5 to 6 μm long and 2.5 to 3.5 μm wide [13]. The acrosome must be well-defined, occupy between 40% to 70% of the total area of the head, and should not contain more than two small vacuoles. The vacuoles must not occupy more than 20% of the area of the sperm head. The acrosomal region stains light blue, while the post-acrosomal region stains dark blue. The post-acrosomal region must not contain any vacuoles [14,33,34]. The mid-piece must be regular, slender, about the same length as the sperm head and stained a purple-red color. The mid-piece must also be aligned

with the axis of the head of the sperm. If a residual cytoplasm larger than one-third of the area of the head is present, the sperm should be considered as abnormal [35]. If present, excess residual cytoplasm may be seen around the midpiece and is be stained pink/red or reddish orange depending on the type of stain used. The tail should be approximately 45 μm long, uniform along its length, appear thinner than the mid-piece, and stained a blue or reddish color. At least 2 replicates of 100 spermatozoa must be scored, with all borderline forms being considered as abnormal. When this strict classification is followed, the reference threshold is ≥4% for morphologically normal forms [13].

QUALITY CONTROL AND QUALITY ASSURANCE IN SPERM MORPHOLOGY ASSESSMENT

Sperm morphology remains one of the most controversial semen parameters, as an incorrect categorization can lead to lack of predictive value for ART outcomes, as reported in several recent publications, although sperm morphology has shown strong predictive values in the early nineties [21-25].

The WHO 5th edition recommends Papanicolaou (gold standard), Shorr and Diff-Quik staining as methods of choice [13]. These methods are adequate for spermatozoa staining and observation under a bright field microscope [36-38]. Any other non-validated staining method can alter the morphological appearance, by causing swelling of the sperm due to osmotic changes [39]. When assessing morphology under bright field optics, spermatozoa in a well-produced and stained smear should show well-differentiated stained regions, such as the acrosomal region, the midpiece, the tail, and the excess of residual cytoplasm, when present. An example of a poor and a good seminal smear staining is shown in Fig. 4 (A and B respectively). Accurate results can be achieved by following a standard operating procedure (SOP) and when the test is performed by a higher-level trained technologist.

QC is crucial in sperm morphology assessment and involves not only the preparation of a good smear, but also a good staining technique. It includes checking the storage conditions, *i.e.* shelf-life, storage temperature, bacterial growth or oily surface film, and changing the stains frequently, depending on the volume/number of slides that have been stained. Reference slides prepared using semen samples of fertile donors or preassessed morphology slides can be used to check the quality of the freshly prepared stains. The quality of the stains should be monitored regularly either biweekly or monthly, according to usage and recorded on the QC forms.

QA is an ongoing process to monitor QC, scoring of morphology, reporting of results, and ensuring low inter- and intraobserver variability. A Bland Altman plot generated with morphology results is used to assess interobserver variability. Validation of sperm morphology results is achieved by testing the samples provided by an external testing agency such as the College of American Pathologists (CAP) in the United States. The laboratory results for sperm morphology analyte need to be within ±2 standard deviation of the mean of the participating laboratories as reported by the CAP. The analytes are tested routinely like other patient samples in the laboratory, and the results are reported using the WHO 5th edition criteria. Corrective action must be taken if laboratory technician's results are out of the range [40]. Among the appropriate measures that could be taken are staff retraining/counseling, recalibration of the instruments, review of the current protocol to identify any potential causes of the discrepancy, and if needed, to implement a change in procedures or protocols, or to institute new laboratory checks. Ongoing monitoring should take place to minimize any recurrence [40,41]. All QA activities, including any problems identified, and corrective actions taken should be docu-

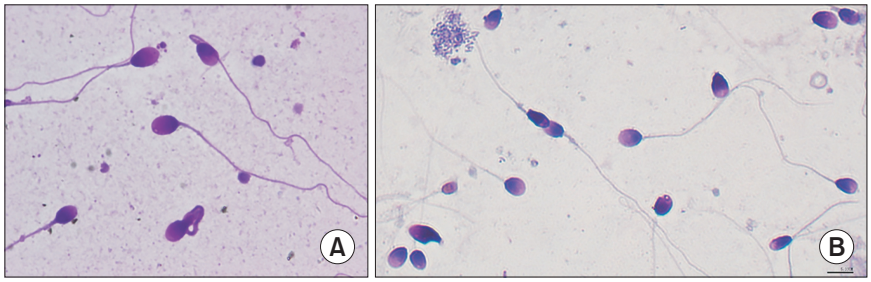


Fig. 4. Example of (A) poor (left side) and (B) good (right side) staining quality.

mented [42].

Proper training of the andrology laboratory staff that follows the guidelines for the strict criteria is the best way to ensure correct evaluation of sperm morphology, uniformity across the laboratory and standardized results. To accomplish this, the Technical Supervisor should perform and document competency assessments through direct observation (side-by-side scoring), by monitoring the recording and reporting of test results, review of intermediate test results or worksheets, direct observation of performing instrument function checks, evaluation of previously tested samples, and assessment of problem-solving skills [43].

CHALLENGES IN MORPHOLOGICAL ASSESSMENT

1. Subjectivity in sperm morphology assessment

Even though sperm morphology is conducted routinely in the general and Andrology laboratories, unlike sperm motility or sperm concentration, it remains a challenge because of the large variability across the staining methods used, or the WHO guidelines followed (1999 *vs.* 2010) by practitioners. In addition, the subjectivity in scoring the sperm can also result from merely observing the sperm and categorizing it as normal or abnormal, without using an ocular micrometer, or confirming potential borderline normal forms with an experienced supervisor to avoid subjectivity. In addition to QC and QA, laboratories need to do an internal audit looking at their morphology scores in their various patient groups and correlate with pregnancies - either natural, IUI, IVF, or ICSI. If they find that a large proportion of their fertile patients are misdiagnosed with teratozoospermia then they would need to look at their criteria for morphology assessment and perhaps revise their scoring accordingly.

2. Differences in technique/protocols in morphology assessment

Staining methods are very important for observing different regions of the spermatozoa under the microscope. Although the Papanicolaou stain is considered the gold standard for sperm morphology, many ART laboratories require a fast turn-around-time for diagnostic reporting of morphology results, and the Papanicolaou protocol can be time consuming. Rapid staining such as pre-stained Testsimplets and Diff-Quik have also been used. The staining procedure for Testsimplets does not require a fixation step and uses a wet mount on special pre-stained slides. Testsimplets however is not recommended by WHO as a standard staining method for the evaluation of sperm morphology [38]. Diff-Quik is a good alternative, which ascertains that results can be reported in a short time.

3. Inter- and intra-observer and laboratory variability

Inter- and intra-observer as well as laboratory variability are some of the challenges in morphological assessment, which can lead to inaccurate results. This can lead to misdiagnosis and mismanagement of the patient. It is important to keep a low inter-observer variability among technicians within the laboratory [44]. With the emphasis on strict morphological criteria, many laboratories appear to have become overly strict in their morphological assessment. As a result, many sperm that are potentially fertile, and therefore "normal", may be labeled as "abnormal", and when the majority of samples are diagnosed as teratozoospermic, the test loses its discriminatory ability. This may be the

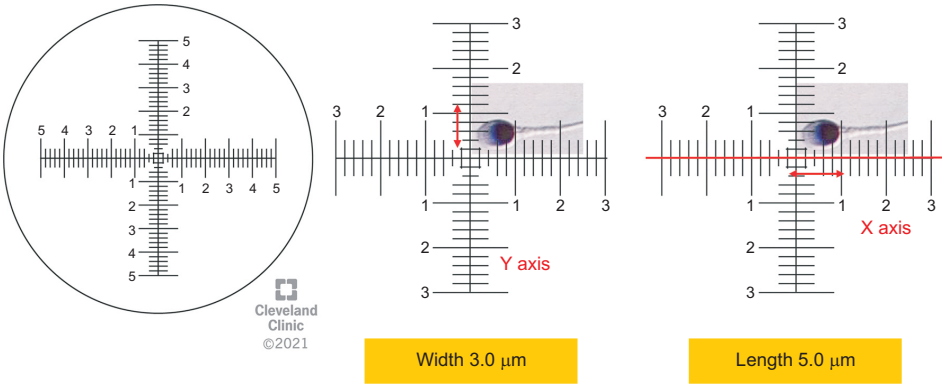


Fig. 5. Schematic representation of how the micrometer is used to measure the dimensions of the sperm head.

reason why several recent studies [25] report that IUI pregnancy rates were unaffected by teratozoospermia. This is indeed a very important issue that needs to be addressed if morphology is to remain a useful predictor of fertility.

The improved predictive power of sperm morphology for ART outcome can be achieved by standardizing the methodology, using an optimal staining method such as Diff-Quik, and an ocular micrometer to measure the sperm dimensions correctly (Fig. 5) and following the WHO 5th edition guidelines for reporting results. The external QC for sperm morphology assessment is met by participating in proficiency testing and performing competency assessments of laboratory personnel. The lab needs to meet the quality standards for internal and external QC and eliminate the inter- and intra-observer variability.

CLINICAL IMPORTANCE OF SPERM MORPHOLOGY ASSESSMENT

In a prospective study with 45 couples undergoing IVF, Kruger et al [14] demonstrated that a threshold of >14% normal sperm morphology was associated with higher fertilization and pregnancy rates. The authors introduced a classification model where they referred to the >14% normal sperm morphology as normal or N-pattern. Patients showing normal morphology rates between 5% and 14% were referred to as good prognosis or G-pattern. In these patients, sperm morphology described by Kruger et al [14] was associated with a significantly higher fertilization rates (%) per oocyte and the average number of embryos per patient (p=0.0001). Sperm morphology of <5% was referred to as poor prognosis or P-pattern. Obara et al [18] examined the relationship between the percentage of normal sperm morphology and fertilization rate in IVF-embryo transfer (IVF-ET) cycles. The fertilization rate (80.5%) in 110 "normal" samples (>14% normal forms) was significantly higher than that (55.4%) in 27 samples with "poor prognosis" (those with 5%–14% normal forms) (p<0.01). The study concluded that ICSI may be required for achieving comparable fertilization rates in samples with a low percentage of normal forms [18].

Sperm morphology according to strict criteria has a strong association with fertilization. Numerous studies have also shown that sperm morphology and total progressive motile sperm count can help in the selection of patients for either IVF or ICSI. In a meta-analysis, which included four retrospective cohort studies covering 2,853 IVF/ICSI cycles, patients with and without severe teratozoospermia (<2% normal forms) (n=673 and 2,183, respectively) were analyzed [22]. Results showed that sperm morphology assessed by strict criteria had little or no prognostic value in ICSI cycle outcomes. However, it should be highlighted that a marked heterogeneity, with a relative risk ranging from 0.40 to 3.36 characterized the ICSI group [22].

A large observational study with 8,846 subjects undergoing 3,676 IVF/ICSI cycles concluded that sperm morphology has poor or no prognostic value in pregnancy outcomes [23]. However, the staining technique employed was not in accordance with the WHO 5th edition standards. Overall, studies assessing the predictive ability of sperm morphology have their own limitations, such as heterogeneity of study groups, staining methods used, scoring and classifications. The importance of morphology is during the initial assessment when consulting with the couple regarding natural conception, IUI or IVF due to the swimming patterns of normal forms. The interaction of the sperm with corona radiata and the sperm head with the oocyte receptors is important to allow acrosome reaction. However, in ICSI, the operator injects a sperm that appears as morphologically normal, unless using the intracytoplasmic morphologically selected sperm injection (IMSI method), where the subjectivity is a huge issue. In ICSI, a spermatozoon is forced into the oocyte independently of its strict morphology (apart from rough head/neck shape and vacuoles), as DNA content/chromatin quality are more important to determine the fate of the embryo. Teratozoospermia should be an independent indication for further clinical investigations as the assumption that poor morphology only requires ICSI misses the opportunity for the diagnosis of male partner. Furthermore, the use of a single sperm in ICSI bypasses natural selection and therefore sperm morphology may contribute to the lack of predictive ability due to this intervention.

Current studies that show the poor predictive ability of sperm morphology have many shortcomings including lack of standardization, deviation from WHO recommended protocols and different outcome measures. Strict criteria classification is based on the concept of natural selection. It can predict sperm fertilizing ability, indicate underlying pathology and be used as a

tool to choose the type of ART method employed. If the percentage of normal forms is ≥4%, then IUI or IVF can be performed. Alternatively, ICSI should be performed when the percentage of sperm with morphologically normal forms is <4%. Standardized morphology assessment by 'strict criteria' remains relevant in making clinical decisions for infertile couples. Future studies should examine the association between sperm morphology, ICSI and birth outcomes.

CLINICAL MANAGEMENT OF PATIENTS WITH ABNORMAL SPERM MORPHOLOGY

Kovac et al [24] reported that natural conception can still occur in up to 30% of men with absolute teratozoospermia (0% normal forms), suggesting that sperm morphology assessment may not be a robust predictor of infertility. On the other hand, various reports have shown significantly higher pregnancy rates in males with normal sperm morphology than that of those with abnormal sperm morphology [27,45,46]. Several studies also established a significant relationship between normal sperm morphology and vital sperm functions including acrosome reaction, binding to the zona pellucida, sperm DNA and chromatin integrity [47-51]. Therefore, treatment directed at improving the percentage of normal sperm morphology is undoubtedly warranted. Furthermore, morphology profiles are characteristic for specific clinical conditions and can therefore provide the clinician with valuable indications for the treatment [52]. Morphological profiles, as seen below, require specific treatments, without which fertilization and conception may not be feasible.

MORPHOLOGICAL PROFILES PROVIDING CLINICAL IMPLICATIONS

1. Globozoospermia

Globozoospermia is a unique sperm morphological defect characterized by round sperm head with the absence of acrosome, and identified in less than 0.1% of male infertility cases [53]. It may be associated with cytoskeletal defects in the sperm as well as sperm DNA fragmentation (SDF) [54-56]. Globozoospermia may be classified as total, with 100% of sperm showing the same defect (Type I), or partial, with the defect found in up to 90% of sperm, and the presence of morphologically normal sperm in semen (Type II). Globozoospermia is frequently found in clusters in certain families denoting its genetic origin [57]. The semen analysis in such cases is usually characterized by a reduction in motility and normal morphology, with or without decrease in sperm concentration [53]. Furthermore, men with globozoospermia may carry a DPY19L2 mutation, which is common in North African men. Globozoospermic spermatozoa are not able to fertilize oocytes, as the acrosomal factor which initiates the oocyte activation, oscillin, is not present in globozoospermic sperm [53]. Moreover, the increased SDF and aneuploidy rates found in globozoospermic sperm decrease their fertilizing ability [53]. This can hinder natural pregnancy as well as the success of ART. ICSI is the only effective treatment in cases of complete globozoospermia, and it should be combined with oocyte activation using calcium ionophore to induce oocyte activation and fertilization [56].

2. Tapered sperm head

Sperm with elongated or pyriform-shaped heads are commonly seen in patients with testicular hyperthermia, commonly secondary to varicocele [58]. This morphological profile may be associated with an increased incidence of tail defects, sperm chromatin abnormalities and SDF, decreasing the fertilizing potential of the sperm [59,60]. These patients may benefit from varicocelectomy or, alternatively, selection of morphologically normal sperm during ICSI [61].

3. Macrozoospermia

Large-headed spermatozoa are defined as those with a length >4.7 μm and a width >3.2 μm [13]. A number of different terms have been used to refer to these spermatozoa, including "macrozoospermia" "macrocephalic sperm", "megalohead" spermatozoa, "enlarged-head spermatozoa" or "enlarged forms" "macronuclear spermatozoa" and "large head spermatozoa".

A high proportion of irregularly shaped, multi-tailed spermatozoa is also associated with severe male factor infertility. High rates of polyploidy and aneuploidy have been described in these cases, in association with Aurora kinase C mutation, frequently seen among North African men [57]. This specific sperm morphology carries a poor prognosis for ICSI, and therefore it is important to document in the semen analysis. Even

in cases of a moderate percentage of large-head sperm, genetic analysis such as sperm FISH could be useful to determine the ploidy of spermatozoa [62].

4. Pin head

A pin head is a spermatozoon lacking chromatin with only a tail [63]. This defect results from errors in the formation of the connecting piece during spermiogenesis. Recent genetic studies related the defect to a mutation in the Sad1 and UNC84 domain containing 5 (*SUN5*) gene, which is normally responsible for the formation of complexes linking the nucleus to the cytoskeleton [64]. Using pin head sperm in ICSI may result in fertilization but with no progression to cleavage-stage embryo [65,66].

5. Residual cytoplasmic droplet

Normal mature spermatozoa do not have a cytoplasmic droplets around the midpiece [67]. In contrast, immature, abnormal sperm have excess cytoplasm that is not removed during spermiogenesis. These are referred to as cytoplasmic droplets and reflect defective maturation processes, and epididymal function associated with infertility [68]. Cytoplasmic droplets are considered an anomaly when they exceed one third of the sperm head size, and they are prevalent in the semen of infertile patients with varicocele [35,69]. Smoking is reported to be associated with increased incidence of cytoplasmic droplets [70]. Antioxidant therapy, varicocelectomy along with lifestyle modification may be beneficial in restoring fertility in such types of sperm morphological abnormalities [71].

6. Tail defects

Tail defects are usually associated with asthenozoospermia. Stump tails and coiled tails are linked to toxic and chemicals substances' exposure [72]. In cases of primary ciliary dyskinesia, sperm tails may appear normal despite total sperm immotility. This is caused by the dynein arm defect in the sperm tail [73]. It is usually associated with chronic respiratory problems and with situs inversus in some cases (Kartagener's syndrome). ICSI can be performed in these cases using ejaculated sperm or testicular sperm. Additional testing such as the hypoosmotic swelling test should be performed to identify viable sperm. Studies have shown that immotile testicular sperm show a better prognosis in ICSI than in the immotile ejaculated sperm [74-76].

GENERAL TREATMENT MODALITIES FOR ABNORMAL SPERM MORPHOLOGY

1. Lifestyle modification and limitation of occupational exposure

Overall, certain lifestyle habits and occupational exposures are known to affect semen quality and fertility potential. However, few studies have investigated the impact of these risk factors on sperm morphology, revealing contradictory results. Pacey et al [77] indicated that modifiable lifestyle factors such as obesity, smoking or alcohol intake/ consumption have little effect on the percentage of sperm morphology. Similarly, Cherry et al [78] examined the occupational exposures of 2,011 men attending infertility clinics across the UK and failed to find any significant relation with sperm morphology. Several other studies reported a significant negative impact of alcohol and smoking on various semen parameters including sperm morphology [79-81]. Similarly, other studies reported a negative impact of exposure to heavy metals (lead, cadmium), bisphenol A, pesticides, radiation, and excessive heat on sperm morphology [82-85]. Regardless of whether lifestyle modification can affect semen quality, adopting healthy habits and avoiding environmental pollutants can positively influence overall health and longevity.

2. Antioxidant supplementation

Antioxidant supplementation is commonly used in the treatment of male infertility to alleviate the detrimental effects of oxidative stress on sperm production [86]. Although of low quality, recent evidence has shown a significant benefit for antioxidant use on conventional semen parameters [86,87]. A systematic review identified the antioxidants vitamin E, N-acetyl cysteine, lycopene, selenium, and zinc to be particularly useful when attempting to address abnormal sperm morphology [88].

3. Varicocelectomy

Varicocele is the most common correctable cause of male infertility. Its detrimental effects on various semen parameters have been established. Treatment is indicated in men with clinically palpable disease in the presence of abnormal semen parameters, including sperm morphology. The current evidence suggests that varicocele ligation significantly improves semen

parameters including morphology. It is also associated with an improvement in fertility potential [89].

4. Assisted reproduction

The use of morphologically normal sperm during ICSI has been shown to overcome most of the sperm abnormalities resulting in better reproductive outcomes. Certain morphological abnormalities such as globozoospermia and primary ciliary dyskinesia require the use of ICSI. Sperm selected for ICSI using ultra-high magnification are reportedly associated with better fertilization and pregnancy rates in couples with male factor infertility or those with a history of ART failure [90].

CONCLUSIONS

In conclusion, evaluation of sperm morphology requires laboratory skills with consistent training and monitoring and the use of an ocular micrometer for accurate measurement of sperm dimensions. Sources of error should be promptly identified, and rectified. QC and QA for sperm morphology are crucial for laboratory accreditation and practices for dependable results. Further, proficiency testing is an external QC tool for reporting accurate morphology results. Although the oocyte and the embryo are key players in ART, spermatozoa equally contribute to a successful outcome. Therefore, the interpretation of sperm morphology is important in the clinical management of infertile couples and in their choice of ART.

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Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: AA, RSharma, SG. Writing – original draft: All the authors. Writing – review & editing: All the authors.

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